

What is claimed is:

CLAIMS

1. A method for screening for one or more nucleic acid sequences that express one or more products that convert a source compound into a target compound, comprising contacting a cell with one or more test nucleic acid sequences, wherein said cell expresses one or more genes encoding one or more proteins that in the presence of said target compound provide a detectable signal, wherein said detectable signal indicates the presence of said one or more nucleic acid sequences.
2. The method of claim 1, wherein said one or more nucleic acid sequences encodes a metabolic pathway not normally present in said cell.
3. The method of claim 2, wherein said one or more nucleic acid sequences are selected from the group consisting of mutagenized DNA, environmental DNA, combinatorial libraries, and recombinant DNA.
4. The method of claim 3, wherein said environmental DNA is isolated from one or more sources selected from the group consisting of mud, soil, water, sewage, flood control channels, and sand.

5. The method of claim 3, wherein said mutagenized DNA is the result of enzyme mutagenesis wherein said mutagenesis is selected from the group consisting of random, chemical, PCR-based, and directed mutagenesis.

5 6. The method of claim 5, wherein said enzyme is selected from the group consisting of lactonases, esterhydrolases, and reductases.

10 7. The method of claim 1, wherein said detectable signal is selected from a group consisting of growth, fluorescence, luminescence, and color.

15 8. The method of claim 7, wherein said detectable signal is growth.

9. The method of claim 1, wherein said target compound provides an element required for growth.

10 10. The method of claim 9, wherein said element is selected from the group consisting of carbon, nitrogen, sulfur, and phosphorous.

11. The method of claim 10, wherein said element is carbon.

12. The method of claim 9, wherein said target compound is selected from the group consisting of ascorbate and 2-KLG.

5 13. The method of claim 12, wherein said target compound is ascorbate.

10 14. The method of claim 1, wherein said source compound is selected from the group consisting of 2-Keto-L-Gulonate, 2,5-Deoxy-Keto-Gulonate, L-Idonate, L-Gulonate, and glucose.

15 15. The method of claim 14, wherein said source compound is 2-Keto-L-Gulonate.

16. The method of claim 1, wherein said cell naturally expresses said one or more genes encoding said one or more proteins that in the presence of said target compound provide a detectable signal.

20 17. The method of claim 16, wherein said one or more proteins are one or more Yia operon-related polypeptides.

25 18. The method of claim 1, wherein said cell has been genetically manipulated to express said one or more genes encoding one or more proteins that in the presence of said target compound provide a detectable signal.

19. The method of claim 18, wherein said one or more proteins are one or more Yia operon-related polypeptides.

5 20. The method of claim 18, wherein said one or more genes encoding said one or more proteins are under the control of an inducible promoter.

10 21. The method of claim 20, wherein said inducible promoter comprises the *trp-lac* hybrid promoter, the *lacO* operator, and the *lacI^q* repressor gene.

15 22. The method of claim 1, wherein said cell grows on ascorbate and does not grow on 2-Keto-L-Gulonate.

20 23. The method of claim 22, wherein said cell is a bacteria.

25 24. The method of claim 23, wherein said bacteria is *Klebsiella oxytoca*.

25 25. The method of claim 1, wherein said cell grows on 2-Keto-L-Gulonate and does not grow on 2,5-Deoxy-Keto-Gulonate.

25 26. An isolated, enriched, or purified nucleic acid molecule encoding one or more Yia operon-related

polypeptides selected from the group consisting of YiaJ, YiaK, YiaL, ORF1, YiaX2, LyxK, YiaQ, YiaR, and YiaS.

27. The nucleic acid molecule of claim 26, wherein
5 said nucleic acid molecule comprises a nucleotide sequence
that:

- (a) encodes a polypeptide having the full length amino acid sequence set forth in SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18;
- (b) is the complement of the nucleotide sequence of (a); and
- (c) hybridizes under highly stringent conditions to the nucleotide molecule of (a) and encodes a naturally occurring polypeptide.

28. The nucleic acid molecule of claim 26, further comprising a vector or promoter effective to initiate transcription in a host cell.

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29. The nucleic acid molecule of claim 26, wherein said nucleic acid molecule is isolated, enriched, or purified from a bacteria.

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30. The nucleic acid molecule of claim 29, wherein said bacteria is *Klebsiella oxytoca*.

31. A nucleic acid probe for the detection of nucleic acid encoding one or more Yia operon-related polypeptides, selected from the group consisting of YiaJ, YiaK, YiaL, ORF1, YiaX2, LyxK, YiaQ, YiaR, and YiaS, in a sample.

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32. The probe of claim 31, wherein said polypeptide is a fragment of the protein encoded by the full length amino acid sequence set forth in SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18.

33. A recombinant cell comprising a nucleic acid molecule encoding one or more Yia operon-related polypeptides selected from the group consisting of YiaJ, YiaK, YiaL, ORF1, YiaX2, LyxK, YiaQ, YiaR, and YiaS.

34. The cell of claim 33, wherein said polypeptide is a fragment of the protein encoded by the amino acid sequence set forth in SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18.

35. An isolated, enriched, or purified Yia operon-related polypeptide selected from the group consisting of YiaJ, YiaK, YiaL, ORF1, YiaX2, LyxK, YiaQ, YiaR, and YiaS.

36. The polypeptide of claim 35, wherein said polypeptide is a fragment of the protein encoded by the full length amino acid sequence set forth in SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18.

37. The polypeptide of claim 35, wherein said polypeptide is isolated, enriched, or purified from bacteria.

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38. The nucleic acid molecule of claim 37, wherein said bacteria is *Klebsiella oxytoca*.

39. An isolated, enriched, or purified nucleic acid molecule, wherein said nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:19.

40. The nucleic acid molecule of claim 39, wherein said nucleic acid molecule comprises:

(a) one or more nucleotide sequences that are set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9;

(b) the complement of the nucleotide sequence of (a);

(c) nucleic acid that hybridizes under stringent conditions to the nucleotide molecule of (a);

(d) the full length sequence of SEQ ID NO:19, except that it lacks one or more of the sequences set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9; and

(e) the complement of the nucleotide sequence of (d).

41. The nucleic acid molecule of either of claims 39
10 or 40, further comprising a vector or promoter effective to
initiate transcription in a host cell.

42. The nucleic acid molecule of claim 41, wherein
said vector or promoter comprises the *trp-lac* hybrid
15 promoter, the *lacO* operator, and the *lacI^q* repressor gene.

43. The nucleic acid molecule of claim 39, wherein
said nucleic acid molecule is isolated, enriched, or
purified from a bacteria.

20 44. The nucleic acid molecule of claim 43, wherein
said bacteria is *Klebsiella oxytoca*.

45. A recombinant cell, comprising the nucleic acid
25 molecule of claim 42.

(d) the full length sequence of SEQ ID NO:19, except that it lacks one or more of the sequences set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9; and

(e) the complement of the nucleotide sequence of (d).

41. The nucleic acid molecule of either of claims 39 or 40, further comprising a vector or promoter effective to initiate transcription in a host cell.

42. The nucleic acid molecule of claim 41, wherein said vector or promoter comprises the *trp-lac* hybrid promoter, the *lacO* operator, and the *lacI^q* repressor gene.

43. The nucleic acid molecule of claim 39, wherein said nucleic acid molecule is isolated, enriched, or purified from a bacteria.

44. The nucleic acid molecule of claim 43, wherein said bacteria is *Klebsiella oxytoca*.

45. A recombinant cell, comprising the nucleic acid molecule of claim 42.

46. A recombinant cell useful for screening for one or more nucleic acid sequences that express one or more products that convert a source compound into a target compound, wherein said cell expresses one or more genes comprising an inducible promoter, and wherein said one or more genes encodes one or more proteins that in the presence of said target compound and an inducer provide a detectable signal, wherein said detectable signal indicates the presence of said one or more nucleic acid sequences.

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47. The recombinant cell of claim 46, wherein said one or more nucleic acid sequences encodes a metabolic pathway not normally present in said cell.

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48. The recombinant cell of claim 47, wherein said one or more nucleic acid sequences are selected from the group consisting of mutagenized DNA, environmental DNA, combinatorial libraries, and recombinant DNA.

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49. The recombinant cell of claim 48, wherein said environmental DNA is isolated from one or more sources selected from the group consisting of mud, soil, water, sewage, flood control channels, and sand.

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50. The recombinant cell of claim 48, wherein said mutagenized DNA is the result of enzyme mutagenesis wherein

said mutagenesis is selected from the group consisting of random, chemical, PCR-based, and directed mutagenesis.

51. The method of claim 50, wherein said enzyme is
5 selected from the group consisting of lactonases,
esterhydrolases, and reductases.

52. The recombinant cell of claim 46, wherein said detectable signal is selected from a group consisting of
10 growth, fluorescence, luminescence, and color.

53. The recombinant cell of claim 46, wherein said detectable signal is growth.

15 54. The recombinant cell of claim 53, wherein said cell requires the presence of said target compound and said inducer for growth.

20 55. The recombinant cell of claim 54, wherein said target compound is selected from the group consisting of ascorbate and 2-Keto-L-Gulonate.

25 56. The recombinant cell of claim 46, wherein said one or more genes are under the control of said inducible promoter.

57. The recombinant cell of claim 56, wherein said inducible promoter comprises the *trp-lac* hybrid promoter, the *lacO* operator, and the *lacI^q* repressor gene.

5 58. The recombinant cell of claim 56, wherein said one or more proteins comprise one or more *Yia* operon-related polypeptides.

10 59. The recombinant cell of claim 58, wherein said cell naturally expresses said one or more genes.

15 60. The recombinant cell of claim 58, wherein said cell has been genetically manipulated to express said one or more genes.

61. The recombinant cell of claim 58, wherein said cell is a bacteria.

20 62. The recombinant cell of claim 61, wherein said bacteria is *Klebsiella oxytoca*.

63. A method for identifying a substance that modulates the conversion of a source compound to a target compound, comprising:

25 contacting a cell with nucleic acid, wherein said nucleic acid expresses a product that converts a source compound into a target compound, and wherein said cell

expresses one or more proteins which in the presence of said target compound provide a detectable signal;

contacting said cell with a test substance; and

monitoring said detectable signal, wherein said

5 detectable signal indicates the presence of said substance.

64. The method of claim 63, wherein the substance is selected from the group consisting of antibodies, small organic molecules, peptidomimetics, and natural products.

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65. The method of claim 64, wherein said detectable signal is selected from a group consisting of growth, fluorescence, luminescence, and color.

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66. The method of claim 65, wherein said detectable signal is growth, and wherein said target compound is metabolizable to an element selected from the group consisting of carbon, nitrogen, sulfur, and phosphorous.

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67. The method of claim 66, wherein said element is carbon.

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68. The method of claim 63, wherein said source compound is selected from the group consisting of 2-Keto-L-Gulonate, 2,5-Deoxy-Keto-Gulonate, L-Idonate, L-Gulonate, and glucose.

69. The method of claim 63, wherein said one or more proteins are one or more Yia operon-related polypeptides.

70. The method of claim 69, wherein said Yia operon further comprises a vector or promoter effective to initiate transcription in a host cell

71. The method of claim 70, wherein said vector or promoter comprises the trp-lac hybrid promoter, the lacO operator, and the lacI^q repressor gene.

72. A method for detecting the presence, absence, or amount of a compound in a sample comprising:
contacting said sample with a cell, wherein said cell expresses one or more genes encoding one or more proteins that in the presence of said compound provide a detectable signal that indicates the presence, absence, or amount of said compound.

73. The method of claim 72, wherein said compound is ascorbate.

74. The method of claim 72, wherein said detectable signal is selected from a group consisting of growth, fluorescence, luminescence, and color.

75. The method of claim 72, wherein said one or more genes comprises *yiaJ*.

76. The method of claim 75, wherein said one or more genes further comprises a promoter transcriptionally linked to a reporter gene.

77. The method of claim 76, wherein *YiaJ* is naturally expressed in said cell.

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78. The method of claim 76, wherein said cell has been genetically manipulated to express said *yiaJ*.

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79. The method of claim 76, wherein the expression of said reporter gene is regulated by the binding of *YiaJ* to said promoter.

80. The method of claim 72, wherein said cell is a bacteria.

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81. The method of claim 80, wherein said bacteria is *Klebsiella oxytoca*.

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82. An isolated, purified, or enriched nucleic acid molecule encoding *YiaJ* and a reporter gene.

83. The nucleic acid molecule of claim 82, further comprising a promoter transcriptionally linked to said reporter gene.

5 84. The nucleic acid molecule of claim 83, wherein the expression of said reporter gene is regulated by the binding of YiaJ to said promoter.

10 85. A recombinant cell for detecting the presence, absence, or amount of a compound in a sample comprising the nucleic acid molecule of either of claims 82 or 83.

15 86. A recombinant cell for detecting the presence, absence, or amount of a compound in a sample, wherein said cell expresses one or more genes encoding one or more proteins that in the presence of said compound provide a detectable signal, wherein said signal indicates the presence, absence, or amount of said compound.

20 87. The recombinant cell of claim 86, wherein said detectable signal is selected from a group consisting of growth, fluorescence, luminescence, and color.

25 88. The recombinant cell of claim 86, wherein said one or more genes comprises *yiaJ*.

89. The recombinant cell of claim 88, wherein said one or more genes further comprises a promoter transcriptionally linked to a reporter gene.

5 90. The recombinant cell of claim 89, wherein YiaJ is naturally expressed in said cell.

91. The recombinant cell of claim 89, wherein said cell has been genetically manipulated to express said *yiaJ*.

10 92. The recombinant cell of claim 89, wherein the expression of said reporter gene is regulated by the binding of YiaJ to said promoter.

15 93. The recombinant cell of claim 86, wherein said cell is a bacteria.

94. The recombinant cell of claim 93, wherein said bacteria is *Klebsiella oxytoca*.

20 95. A method of selection for a nucleic acid sequence encoding a metabolic pathway from a source compound to a target compound comprising:

25 (1) identifying an organism that metabolizes a target compound to provide an essential element;

(2) identifying one or more genes responsible for the metabolism of said target compound to said essential element;

5 (3) expressing said one or more genes under the control of an inducible promoter, whereby said target compound is metabolized in the presence of an inducer and not in the absence of said inducer;

10 (4) expressing nucleic acid sequences potentially encoding said metabolic pathway in said recipient organism; and

15 (5) selecting said recipient organism for growth on said source compound in the absence of said target compound and in the presence of said inducer, wherein growth on said source compound in the absence of said target compound and in the presence of said inducer indicates the presence of said nucleic acid sequence.

96. The method of claim 95, wherein said essential element is selected from the group consisting of carbon, phosphorous, nitrogen, and sulfur.

97. The method of claim 96, wherein said essential element is carbon.

25 98. The method of claim 95, further comprising the transfer of said one or more genes to a highly genetically manipulatable recipient organism, such that said recipient

organism metabolizes said target compound to provide an
essential element.

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